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## Activities of Taurolidine In Vitro and in Experimental Enterococcal Endocarditis

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### ► ABSTRACT

In vitro, the antimicrobial agent taurolidine inhibited virtually all of the bacteria tested, including vancomycin-resistant enterococci, oxacillin-resistant staphylococci, and *Stenotrophomonas maltophilia*, at concentrations between 250 and 2,000 µg/ml.

Taurolidine was not effective in experimental endocarditis. While it appears unlikely that this antimicrobial would be useful for systemic therapy, its bactericidal activity and the resistance rates found ( $<10^{-9}$ ) are favorable indicators for its possible development for topical use.

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### ► TEXT

With the continuing emergence of multiply antibiotic-resistant organisms, the need to develop new therapeutic agents remains evident. Taurolidine [bis-(1,1-dioxoperhydro-1,2,4-thiadiazinyl-4)methane], a derivative of the amino acid taurine, is an antimicrobial agent which inhibits and kills a broad range of microorganisms in vitro, albeit at high concentrations (3, 4, 9, 11, 13). This compound acts through mechanisms unlike those described for other currently available antimicrobials. Specifically, it is believed that methylol derivatives interact with components of bacterial cell walls resulting in irreparable injury (4). Taurolidine also appears to have immunoregulatory properties, blunting lipopolysaccharide-induced tumor necrosis factor and interleukin-1 release from human peripheral blood mononuclear cells (2) and also reducing adherence of

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bacteria to human epithelial cells in vitro (5). The compound has been given to humans both intravenously (i.v.) and by peritoneal lavage (1, 12).

The purpose of the present study was to examine the in vitro activity of taurolidine against a broad variety of bacterial species, including antibiotic-resistant strains. We also evaluated the activity of taurolidine in vivo in experimental endocarditis using two strains of enterococci, one of which was a vancomycin-resistant strain of *Enterococcus faecium*.

Most of the bacterial strains used in this study were routine isolates collected by our clinical microbiology laboratory during 1997. Additional strains from our collection were included based upon specific resistance traits. Taurolidine was provided by Wallace Laboratories, Cranbury, N.J. Antimicrobial reference standards of ciprofloxacin, imipenem, and cefotaxime were provided by Bayer Corporation, West Haven, Conn.; Merck & Co., Inc., West Point, Pa.; and Hoechst Marion Roussel, Inc., Kansas City, Mo., respectively. Vancomycin was obtained from Eli Lilly & Co., Indianapolis, Ind. MICs were determined by agar dilution (7, 8) on Mueller-Hinton II agar (BBL Microbiology Systems, Cockeysville, Md.) except as noted otherwise. Agar was supplemented with 5% sheep blood for streptococci and diphtheroids. Inocula were ca.  $10^4$  ( $10^5$  for anaerobes) CFU/spot. Plates were incubated in room air and read at 18 to 20 h, except for lactobacilli, *Leuconostoc* spp., *Pediococcus* spp., and pneumococci, which were incubated in 5% CO<sub>2</sub> and examined for growth at 24 h. Anaerobes were incubated for 48 h on brucella agar in an atmosphere produced by Gas-Pak Plus (BBL). Time-kill studies were carried out with Mueller-Hinton broth with no antibiotic or with taurolidine at the MICs and four times the MICs for individual strains. No attempt was made to inactivate or remove the antimicrobial, except by dilution. To test for the emergence of resistant subpopulations, suspensions of organisms grown overnight in broth were concentrated fivefold and 0.1 ml of each suspension was laid onto the surface of an agar plate containing taurolidine at two and four times the MIC for each organism. The plates were examined for growth at 48 h of incubation.

Experimental endocarditis was established as described previously (6). Two enterococcal strains were used in these experiments: vancomycin-susceptible strain *E. faecalis* 1310 and vancomycin-resistant (VanA) strain *E. faecium* A1221. The characteristics of these organisms have been described recently (10). Mean injected inocula were  $2.2 \times 10^7$  and  $1.1 \times 10^9$  CFU, respectively. Treatment was started 24 h after inoculation and continued for 5 days. Taurolidine or a placebo (excipients only) was delivered by continuous i.v. infusion via an indwelling central venous catheter. Taurolidine was given intravenously at a dose of 720 mg/kg/day, which was the maximum feasible dose, given the formulation. In several experiments, i.v. taurolidine was supplemented with intraperitoneal (i.p.) administration of 2 ml of a 2% solution of taurolidine in saline (total, 40 mg/dose) twice daily, yielding a total daily dose (i.v. plus i.p.) of ca. 1,120 mg/kg. Animals were sacrificed approximately 3 h after discontinuation of i.v. infusions. For animals receiving i.p. injections, the last dose was given 14 h before sacrifice. Aortic valve vegetations were aseptically removed, homogenized, and serially diluted in sterile saline for bacterial colony counts. Only animals having received at least 4 days of therapy and with correct placement of the aortic valve catheter determined at necropsy were included in the evaluation.

Agar dilution MICs of taurolidine and comparison agents are shown in Table 1. Virtually all of the

organisms tested were inhibited by taurolidine at concentrations between 250 and 2,000 µg/ml. Included among the enterococci were 43 vancomycin-resistant *E. faecium* and 21 vancomycin-resistant *E. faecalis* isolates. Activity of taurolidine against oxacillin-resistant staphylococci (including two glycopeptide-intermediate *S. aureus* isolates) was equivalent to that against oxacillin-susceptible strains. Occasional strains of gram-positive bacteria, including all 10 strains of *Clostridium difficile*, were inhibited at 125 µg/ml, while two strains of *Burkholderia cepacia* were inhibited only at 4,000 µg/ml.

**TABLE 1.** Comparative in vitro activities of taurolidine and other antimicrobials

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At 2,000 µg/ml, taurolidine was bactericidal against five of the six isolates shown in Table 2 and against both of the strains used in the endocarditis model. Killing over 24 h was also seen at 500 µg/ml against one strain each of *Escherichia coli* and *Staphylococcus aureus* and against *E. faecium* A1221 (killing, 3.1 log<sub>10</sub> CFU/ml) but not *E. faecalis* 1310 (killing, 1.3 log<sub>10</sub> CFU/ml). Plating of large inocula of several strains on agar containing taurolidine at 1 or 2 mg/ml failed to yield growth of subpopulations or mutants resistant to taurolidine. Resistance rates were <10<sup>-9</sup> at two and four times the MIC for two strains each of *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *E. faecalis*, and *E. faecium*.

**TABLE 2.** Taurolidine killing activity against selected organisms as defined by reduction in the number of viable cells from the inoculum colony count

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Taurolidine was ineffective in experimental enterococcal endocarditis (Table 3). In Sprague-Dawley rats, i.p. injections of taurolidine resulted in unexpectedly high mortality (60%) and surviving animals had extensive peritoneal inflammation, with exudate and intestinal adhesions. Because of the inflammatory changes seen with i.p. taurolidine during experiments with *E. faecium* A1221 in Sprague-Dawley rats, only i.v. taurolidine was studied against *E. faecalis* 1310 in this strain of rat. Wistar rats tolerated i.p. taurolidine injections with no increased mortality or peritoneal abnormalities noted at necropsy. Nevertheless, the combination of i.v. plus i.p. taurolidine was ineffective against infection due to *E. faecium* A1221 in this strain of rat as well.

**TABLE 3.** Activity of taurolidine in experimental enterococcal endocarditis

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Our study confirmed the in vitro activity of taurolidine against a broad range of gram-positive and gram-negative organisms including oxacillin-resistant *S. aureus* and coagulase-negative staphylococci,

vancomycin-resistant enterococci, and gram-negative problem pathogens, including *P. aeruginosa* and *Stenotrophomonas maltophilia*. MICs of taurolidine (250 to 1,000 µg/ml) against vancomycin-resistant enterococci and methicillin-resistant *S. aureus*, including glycopeptide-intermediate *S. aureus* strains, encompassed the range of MICs recently reported against a small number of isolates of these groups (L. A. Mermel, N. Magill, and S. Zinner, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-190, 1998; L. A. Mermel, N. Magill, and S. Zinner, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-191, 1998). Although the MICs of taurolidine were high, the agent has been reported to be relatively nontoxic. The 50% lethal dose for rats exceeds 4,000 mg/kg (data from Wallace Laboratories). To determine whether taurolidine activity could be demonstrated in vivo in our experimental model, we employed the maximum doses which could be physically administered with combined i.v. plus i.p. dosing. Even with such doses, we were unable to show activity in vivo against either test organism in this model.

It was a limitation of this study that we were unable to determine concentrations of taurolidine or its metabolites in the plasma of treated animals. Nevertheless, it seems likely that the combined levels of taurolidine and its metabolites, taurinamide and taurultam, exceeded concentrations inhibitory against the test organisms for some period of time. In rats given radiolabeled taurolidine, after doses of 100 mg/kg i.v. or i.p., peak concentrations in plasma reach 100 to 200 µg/ml and levels exceed 30 µg/ml for at least 2 h (data from Wallace Laboratories). With combined therapy, rats in our experiments received more than 10 times that dose.

Because peak concentrations of taurolidine and its metabolites determined to date in the plasma of humans do not appear to reach the MICs against many of the strains of concern, it seems doubtful that this drug would have a significant role in the systemic therapy of established infections. On the other hand, the bactericidal activity of this agent and the low resistance frequencies found are favorable indicators for the possible development of taurolidine for topical or local use. This would be especially true if its activity is retained on mucosal surfaces, on the surfaces of catheters and prosthetic devices, in flushing solutions, or in various body fluids.

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## ► FOOTNOTES

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